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Competition for Space Among Sessile Marine Invertebrates: Changes in HSP70 Expression in Two Pacific Cnidarians

SERGI ROSSI* AND MARK J. SNYDER†

University of California, Davis, and Bodega Marine Laboratory, P.O. Box 247, Bodega Bay, California 94923

Abstract. The role of stress proteins—either constitutive (HSC) or inducible (HSP)—of the HSP70 family in intra- and interspecific competition for space was examined in two sessile Pacific cnidarians. *Anthopleura elegantissima,* an intertidal anemone, and *Corynactis californica,* a subtidal corallimorpharian, express HSP70 in the absence of apparent physical stress. HSP70 protein expression is concentrated in the tentacles of *A. elegantissima* when the animal is exposed to contact with other benthic organisms. Under the same conditions, however, HSP concentrations are similar in the body and tentacles of *C. californica.* When two different clones of *A. elegantissima* interact in the field, the outside polyps (warriors) express more HSP70 than the inside ones (2.4 *versus* 0.6 ng HSP70/μg Protein). When different *C. californica* clones interact, HSP70 expression in the outside and inside polyps is similar (1.5 *versus* 1.8 ng HSP70/μg P) and is fairly constant in the corallimorpharian in the different interspecific encounters. HSP70 expression is related to the different kinds of aggression encountered by both cnidarians. HSP70 expression may be involved in the recovery of tissues damaged by the allelochemical, cytotoxical, or corrosive substances produced by different enemies. *C. californica* clones appear prepared for war, as evidenced by the high constant expression of HSP70 in the polyps. *A. elegantissima* exhibits differential HSP70 expression depending on the identity of each neighboring intra- or interspecific sessile competitor. We propose that stress proteins can be used to quantify space competition or aggression among sessile marine invertebrates.

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* Current address: Institut de Ciènces del Mar, Passeig Nacional, s/n, 08003 Barcelona, Spain.

† To whom correspondence should be addressed. E-mail: mjsnyder@ucdavis.edu

Introduction

Space on which to live is often the most limiting resource in marine hard-substratum environments, and patchiness has evolved under the influence of intense competition for living space (Connell, 1961; Pequegnat, 1964; Paine, 1971; Dayton, 1971; Jackson, 1977). Once established, organisms can show aggressive behavior (Chadwick, 1987) that may be especially intense in cryptic environments where free space is almost nonexistent.

In benthic environments, sponges, ectoprocts, cnidarians, and ascidians can produce biologically active substances that may be destructive to enemies during space competition (Whittaker and Feeny, 1973; Uriz *et al.,* 1991). These organisms aggregate in patches that can dominate hard-bottom substrates (Sutherland, 1978; Chornesky, 1983; Chadwick, 1987, 1991; Chadwick and Adams, 1991; Langmead and Chadwick, 1999a, b, among others). Growth is often slow in such organisms, and interactions between competitors are often nonevident. It is difficult to quantify competitive interactions *in situ,* and the manipulation of organisms is frequently essential to demonstrate the potential effects of space competition (Schoener, 1983). For example, investigators have rarely observed agonistic interactions in wild anemones (*A. xanthogrammica*), although these organisms frequently exhibit such behavior in forced situations (Sebens, 1984). The quantification of damage from encounters between such organisms and the identification of potential mechanisms used to counter the effect of such aggression have proved difficult. Most studies have dealt with the organismal responses to the attack and the

consequent aggressive behavior displayed by individuals. Few workers have focused on the capacities, and implied mechanisms, for tissue recovery following aggressive interactions. We hypothesize that components of the stress response such as HSPs may provide evidence of the intensity of competitive interactions and are one of the mechanisms by which cnidarians recover from or prepare their tissues for the effects of competitive or aggressive interactions.

HSPs enhance cell survival by reducing the accumulation of damaged or abnormal polypeptides within cells (Feder and Hofmann, 1999). However, whether all wild organisms routinely, occasionally, or seldom express inducible HSPs is unknown. For marine invertebrates, most investigators have examined the effects of thermal variations on constitutive (HSC70) and inducible (HSP70) responses (Feder and Hofmann, 1999). Competitive interactions between sessile organisms can elicit HSP responses due to protein damage following the excretion of harmful substances by one or both competitors (Uriz *et al.*, 1991; Turon *et al.*, 1996; Wiens *et al.*, 1998). One index of tolerance to aggressive sessile organisms could be the presence and abundance of mechanisms (such as HSPs) that would resist or ameliorate the damage inflicted on cellular components by the potential space competitor. Furthermore, once HSP can be related to space competition, no manipulation will be necessary to test such hypotheses. HSP expression could then be a quantitative tool to examine competitive interactions in the field without human interference.

To determine whether HSP expression patterns could be related to competitive interactions in marine hard-bottom sessile invertebrates, two Pacific cnidarians were chosen for study: the intertidal anemone *Anthopleura elegantissima* and the subtidal corallimorpharian *Corynactis californica*. *A. elegantissima* forms contiguous aggregations composed of individuals of a single clone, the products of asexual reproduction (Francis, 1973b; Sebens, 1982a, b). Free zones are created where competition between clones occurs through the outside polyps of the aggregation (called "warriors," Francis, 1973a). Compared with polyps in the center of the clone, the warriors have larger and more abundant acrorhagi (specialized nonfeeding tentacles) and lack mature gonads (Francis, 1973b, 1976). The aggressive response is not directly involved in either defense against predators or capture of prey (Francis, 1973b), but functions in the competition for space. We hypothesize that *A. elegantissima* warriors may exhibit higher HSP levels than interior clonemates because they interact more frequently with competitors.

In the subtidally distributed *C. californica*, the polyps have no distinctive roles within each clone (Chadwick, 1987). Although the physiology of this group is not as well understood as that of anemones, several studies have described the competition for space and the specific responses to aggression in corallimorpharians (Chadwick, 1987, 1991; Chadwick and Adams, 1991; Langmead and Chadwick, 1999a, b). Space competition experiments demonstrate that *C. californica* influences the abundance and population structure of other cnidarians by means of its aggressive behavior (Chadwick, 1987, 1991; Chadwick and Adams, 1991). We sought to determine whether the high aggression in this species is related to elevated HSP levels as preparation for possible damage resulting from such interspecies encounters.

We tested two main hypotheses in this work: first, that stress produced by space competition can induce HSP expression to counter the effects of aggressive neighbors; second, that HSP expression can provide a quantitative assay for space competition in sessile invertebrates.

Materials and Methods

Animals and treatments

Anthopleura elegantissima and *Corynactis californica* were collected from the Bodega Bay area and held in the running seawater system of the Bodega Marine Laboratory. All animals were held in ambient seawater (13–15 °C) and fed adult brine shrimp or frozen seafood. The seawater from the Bodega Bay area is considered clean, and the animals used in these experiments are considered to have had minimal contact with anthropogenic chemicals that are known to induce HSP expression (McCain *et al.*, 1988). All experiments (aquarium and field) were done in September–October 1998 and 1999 to avoid seasonal differences in cnidarian behavior. Each experiment, whether forced interactions in an aquarium or *in situ* interaction, was designed to assess the effects of neighboring competition for space on HSP70 expression.

Forced aquarium experiments

The first experiment examined HSP70 protein expression in *A. elegantissima* and *C. californica* in a forced situation. Six isolated polyps of each species (attached to stones, no physical stress induced) were moved into contact with each other (*i.e.*, one polyp of *A. elegantissima* against one polyp of *C. californica*). After 24 h, tentacle samples from three individuals of each species were removed and frozen in liquid nitrogen. To quantify the differences between tentacles and body, the other three polyps of each species were sampled 48 h later, frozen in liquid nitrogen, and then assayed for HSP70 level by methods detailed below. As controls, isolated polyp tentacles (n = 5–6, no interacting species) of *A. elegantissima* and *C. californica* were likewise sampled in the aquarium.

In situ *intraspecific competition*

We assessed HSP70 expression related to competition for space in a natural environmental situation (*i.e.*, in natural

clones in the field). Because collection and transport of animals to artificial holding conditions can stimulate a stress response (Sharp *et al.*, 1994; Roberts *et al.*, 1997), clones of *A. elegantissima* and *C. californica* were located and sampled from the Bodega Bay Jetty from a minimum 2 m below the 0 tide level (permanently submerged). This avoided significant desiccation, changes in temperature, fluctuations in salinity and pH, and other effects that are typical of the environment for the intertidal *A. elegantissima* but not for the subtidal *C. californica*.

For the *A. elegantissima* intraspecific competition experiments, clones were located by scuba and photographed (Nikonos V camera, 35-mm lens with macro 1:1 or close-up lens). Polyps of each clone were sampled ($n = 3$, tentacles) from the outside (touching the competitor) and the inside (touching only the same clone, 10–20 cm from the outside polyps). Samples were dissected, kept in 13 °C seawater for no longer than 30 min before freezing in liquid nitrogen, and stored at −70 °C. As a control to assess whether HSP70 levels were affected by the extra 30-min tissue incubation in ambient seawater before freezing, the following experiment was performed. Individual tentacle samples were obtained from three individuals of two clones exposed to elevated temperatures in the intertidal zone (elevated HSP70 is found in these conditions, Snyder and Rossi, unpubl. obs.). Each sample was divided into three parts, of which two were immediately frozen in liquid nitrogen and the third was submerged in ambient seawater for 40 min prior to freezing as above.

For the *C. californica* intraspecific competition experiments, six clones were located and sampled as above. Color varies greatly between different clonal aggregations, which is useful in distinguishing clones that show potential intraspecific competition. Outside and inside polyps (tentacle crowns) of each clone were sampled to compare interacting (<2.5 mm apart) and non-interacting individuals (5–10 cm apart from the outside ones).

Interspecific competition

To examine the effects that different space competitors in the benthic substrata have on HSP70 protein levels, we chose two genera of algae that compete for space with *A. elegantissima* and *C. californica* and two intertidal and two subtidal invertebrates for *A. elegantissima* and *C. californica*, respectively. The sampled and photographed anemone clones were always submerged (as described before).

Four clones of *A. elegantissima* and three of *C. californica* that were interacting with a calcareous red alga (*Lithothamnium* sp.) were dissected (outside and inside clone tentacles). Another alga interacting with both cnidarians was a fleshy green alga (*Ulva* sp.), and six clones of each cnidarian were sampled as above.

In the high subtidal, common space competitors of *A. elegantissima* are the anemone *A. xanthogrammica* and the cirriped *Balanus amphitrite*. Five *A. elegantissima* clones interacting with *A. xanthogrammica* were sampled in the outside and inside parts of the clones. For *B. amphitrite*, three clones competing for space were likewise sampled. For *C. californica*, the subtidal organisms chosen (sponge *Haliclona permollis;* ascidian *Synoicum parfustis*) were considered potentially more aggressive than the fleshy algae. Six *C. californica* clones were chosen for their clear interactions with *H. permollis*, and polyps of the outside and inside part of each clone were dissected. For *S. parfustis*, the interaction of the clones was observed in four populations in the dive area, and outside and inside polyps were sampled.

HSP70 measurements

The western immunoblotting for HSP70 expression was done as follows. Frozen tentacle samples (stored at −70 °C) were individually homogenized in 0.2 ml of buffer K containing 5 m*M* $NaHPO_4$, 40 m*M* HEPES (pH 7.4), 5 m*M* $MgCl_2$, 70 m*M* potassium gluconate, 150 m*M* sorbitol, and 1% SDS. Homogenates were centrifuged 10 min at 10,000 × *g*, and the supernatants were combined with equal volumes of SDS sample buffer (Laemmli, 1970) and boiled for 5 min. Supernatant protein levels were determined by BioRad DC assay, and 20 μg of tentacle protein was loaded in each gel lane. For each blot, 50 ng of standard HSP70 protein (human, StressGen) was included. Discontinuous SDS gels (1 mm) were 6.2% for the stacking gel and 12% for the resolving gel. After running for 2 h at 150 V, SDS gels were electroblotted onto PDVF membranes (for 1 h at 100 V). The protein bands in each western blot were visualized by staining with Ponceau S. HSP70 protein was detected with mouse monoclonal anti-HSP70 (SPA-822, StressGen, Victoria, BC); the secondary antibody was goat-anti-mouse IgG, conjugated to peroxidase (Sigma), and was visualized with ECL reagents (Amersham) and exposure of blots to X-ray film.

Blot band intensities were compared by scanning the X-ray films and analyzing the scans with the NIH Image software package. For each blot, the scanned intensity of the HSP was normalized against the intensities of the HSP70 protein standard from that blot; that is, the NIH Image datum point was divided by the intensity of the HSP70 standard.

Results

Anthopleura elegantissima and *Corynactis californica* express a single HSC70 or HSP70 protein (Fig. 1). In other eukaryotes, the HSP70-DnaK protein family comprises multiple proteins, more than one of which may be detected by the antibody. For the sake of convenience, we will collectively term these as "HSP70." The inclusion of protease inhibitors did not affect HSP70 levels (Fig. 1A,

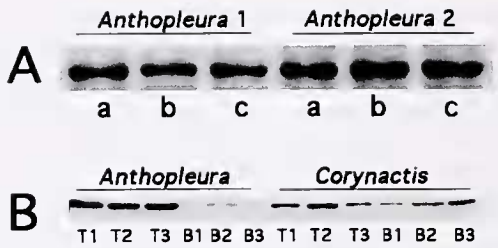

Figure 1. Western blots of HSP70 levels in *Anthopleura elegantissima* and *Corynactis californica:* comparison of tentacles under different sampling conditions and body without tentacles. In (A), triplicate tentacle samples were taken from two *A. elegantissima* individuals; (a) the first of the triplicate samples was immediately frozen in liquid nitrogen, (b) duplicate of (a) with the addition of protease inhibitors prior to homogenization, and (c) third sample from each anemone kept in a sample bag submerged at 13 °C for 40 min prior to freezing in liquid N_2. In (B), three individuals from each species were divided into tentacles only or body minus tentacles prior to homogenization.

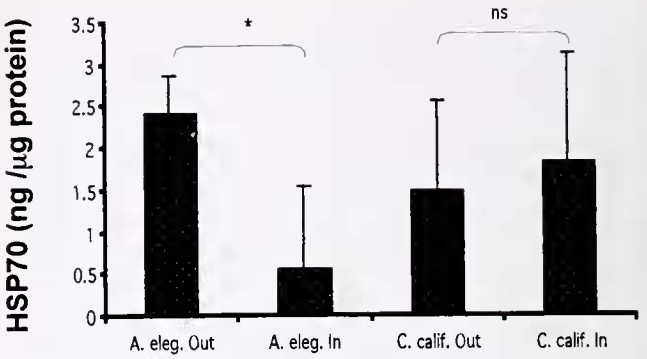

Figure 3. Intraspecific competition. HSP70 expression between tentacles of the inside and outside polyps in *Anthopleura elegantissima* and *Corynactis californica* in intraspecific conditions. The bars are + 1 standard deviation of 4–5 clones. Asterisks indicate significant differences between groups ($P \leq 0.05$); ns indicates a lack of significant differences between groups.

Anthopleura 1 and 2, a *versus* b); therefore they were omitted from our studies during the homogenization steps. The 30-min ambient seawater submersion of subtidal tentacle samples prior to freezing had no effect compared with immediate freezing (Fig. 1A, *Anthopleura* 1 and 2, c *versus* a and b). In comparing tentacles of the same polyp 24 h after the first forced interaction between the two cnidarian species in the laboratory, no differences were observed ($F(3, 8) = 2.0$, $P < 0.1929$) (Fig. 2). Two days later, HSP70 levels in *A. elegantissima* tentacle were 4 times greater than before (4.0 ± 0.5 ng HSP70/µg P in the tentacles; 0.0 ± 0.1 ng HSP70/µg P in the body, power of test = 0.87), but no differences were detected in *C. californica* tentacles (1.7 ± 0.9 ng HSP70/µg P in the tentacles; 0.8 ± 0.9 ng HSP70/µg P in the body) (Fig. 2). Differences between tentacles and body were found in *A. elegantissima* but not in *C. californica* (Fig. 1; $F(3, 8) = 18.55$, $P < 0.0006$, power of test = 0.98). Algal symbionts are at the highest concentration in *A. elegantissima* oral disk (Fitt *et al.*, 1982; Weis and Levine, 1996); these data imply that we are measuring HSP70 responses in animal tissue. No such differences were found in the corallimorpharian, which lacks algal symbionts.

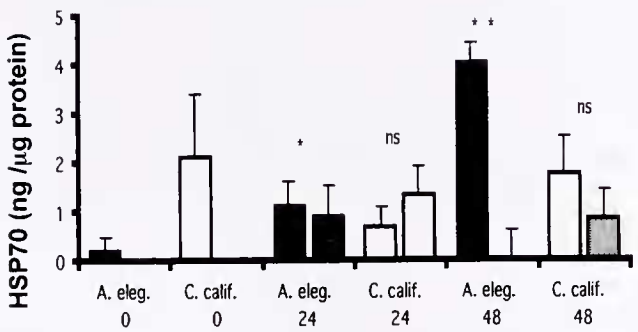

Figure 2. HSP70 expression in tentacles of *Anthopleura elegantissima* and *Corynactis californica* at time 0, 24 h after the first contact of the cnidarians (A. eleg. clones 1 and 2, black and stippled; C. calif. Clones 1 and 2, white and stippled), and 48 h later in both tentacles and body (without tentacles, stippled) of the same polyps in *A. elegantissima* and *C. californica.* The bars are +1 standard deviation of 3–6 samples. Asterisks indicate significant differences between groups ($P \leq 0.05$); ns indicates a lack of significant differences between groups.

HSP70 levels in isolated polyps were also examined under the same conditions (no contact with any other invertebrate). *A. elegantissima* tentacles had very low expression (0.2 ± 0.3 ng HSP70/µg P) compared with the previous contact experiments. *C. californica* had high expression (2.1 ± 1.3 ng HSP70/µg P) even when there was no direct (contact) aggression present. Comparing this analysis with the anemone-corallimorpharian experiments, no differences were found between HSP70 expressions in *C. californica.* There were differences in the HSP70 expression of polyps between the two cnidarians when they were compared together ($F(1, 9) = 10.81$, $P < 0.0094$).

The mean distance between competitors in field studies as determined from the photographs was 2.4 ± 0.9 mm ($n = 17$). This distance is clearly within the range that *A. elegantissima* tentacle crowns sway during seawater movements (Francis, 1973a). The results of intraspecific competition in selected patches of both cnidarians are shown in Figure 3. There were clear differences in *A. elegantissima* HSP70 expression between the outside warrior polyps and the inside ones (in contact, 2.4 ± 0.5 ng HSP70/µg P; no contact, 0.6 ± 0.7 ng HSP70/µg P; $F(3, 20) = 3.93$, $P < 0.0234$, power of test = 0.82) when two clones of the same species interacted. Interestingly, *C. californica* had similar HSP70 amounts in polyps of different clones (outside 1.5 ± 1.1 ng HSP70/µg P; inside 1.8 ± 1.3 ng HSP70/µg P).

The regular cnidarian HSP70 expression in both outside and inside polyps of the clone in different competition-for-space situations is illustrated in Figure 4. *A. elegantissima*

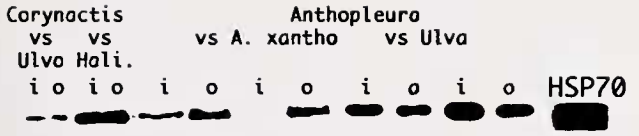

Figure 4. Western blot of HSP70 levels in *Anthopleura elegantissima* and *Corynactis californica* tentacles from inside not interacting (i) and outside interacting (o) analyzed with competitors in the field. *C. californica* competitors were *Ulva* sp. and *H. permollis*. *A. elegantissima* competitors were *A. xanthogrammica* and *Ulva* sp.

had more HSP70 in the warriors than in the inside clone polyps in general, depending on the competing species (Fig. 4). In Figure 5A, B we show HSP70 levels when both cnidarians interacted with the same competitors in the field: crustose red (*Lithothamnium* sp.) and fleshy green (*Ulva* sp.) algae. Contact with *Lithothamnium* (Fig. 5A) resulted in higher HSP70 expression in the outside *A. elegantissima* clone polyps (warriors, 2.4 ± 1.2 ng HSP/μg P; inside ones 0.5 ± 0.4 ng HSP/μg P, $F(3, 10) = 4.82$, $P < 0.025$, power of test = 0.80). No differences were found between the inside and outside *C. californica* polyps in interactions with either algal species (outside 1.2 ± 0.4 ng HSP/μg P; inside 1.5 ± 0.5 ng HSP/μg P).

Neither cnidarian showed any significant difference in HSP70 between inside and outside polyps (Fig. 5B). In *A. elegantissima*, the inside polyps (1.0 ± 0.8 ng HSP70/μg P) were similar to the outside ones (0.6 ± 0.6 ng HSP70/μg P). The expression was also similar for both clone polyps in *C. californica* (outside 1.2 ± 0.6 ng HSP70/μg P; inside 1.3 ± 0.8 ng HSP70/μg P). *C. californica* HSP70 expression was always the same in the outside and inside polyps (1–1.8 ng HSP70/μg P) in encounters with either *A. elegantissima*, other *C. californica* clones, or either algal species.

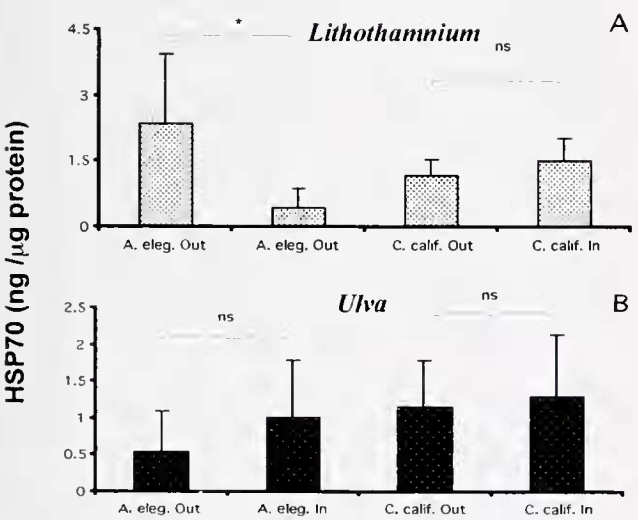

Figure 5. Interspecific competition I. HSP70 expression between tentacles of the inside and outside polyps in *Anthopleura elegantissima* and *Corynactis californica* in contact with calcareous red (*Lithothamnium* sp.) (A) and fleshy green (*Ulva* sp.) (B) algae. The bars are +1 standard deviation of 4–6 clones. Asterisks indicate significant differences between groups ($P \leq 0.05$); ns indicates a lack of significant differences between groups.

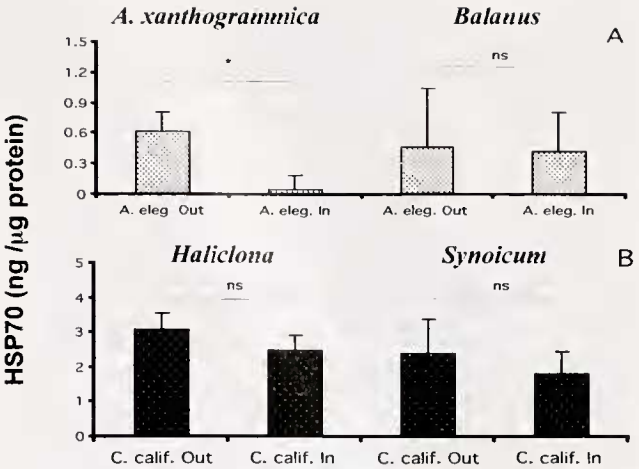

Figure 6. Interspecific competition II. HSP70 expression between tentacles of the inside and outside polyps in *Anthopleura elegantissima* and *Corynactis californica* with different competitors. (A) *A. elegantissima* against *A. xanthogrammica* and *Balanus*; (B) *C. californica* against *Haliclona permollis* and *Synoicum parfustis*. The bars are +1 standard deviation of 3–5 clones. Asterisks indicate significant differences between groups ($P \leq 0.05$); ns indicates a lack of significant differences between groups.

For *A. elegantissima*, two intertidal competitors were tested in submersed conditions: *A. xanthogrammica* and *Balanus amphitrite* (Fig. 6A). Encounters with *A. xanthogrammica* resulted in higher HSP70 in *A. elegantissima* outside polyps (0.6 ± 0.2 ng HSP70/μg P; inside ones 0.1 ± 0.1 ng HSP70/μg P, $F(3, 12) = 2.88$, $P < 0.048$, power of test = 0.99). However, HSP70 levels were low compared with other situations (interactions with calcareous algae or other *A. elegantissima* clones). No differences in HSP70 level were found with the *B. amphitrite* interactions (outside 0.5 ± 0.6 ng HSP70/μg P; inside 0.4 ± 0.4 ng HSP70/μg P).

Differences in *C. californica* HSP70 levels occurred when potential encounters and fights for space were against the sponge *Haliclona permollis* or the ascidian *Synoicum parfustis* (Fig. 6B). HSP70 expression was the same in the outside and inside polyps, but was slightly higher than with other competitors. Both sponge and ascidian appear to activate higher HSP70 expression (*H. permollis* outside 3.1 ± 0.5 ng HSP70/μg P; inside 2.5 ± 0.5 ng HSP70/μg P; *S. parfustis* outside 2.4 ± 1.0 ng HSP70/μg P; inside 1.8 ± 0.6 ng HSP70/μg P). Again, no significant differences were found between inside and outside polyps. When comparing the response of this cnidarian against the sponge and the ascidian with all the other encounters, significant HSP70 differences were found ($F(5, 79) = 18.58$, $P < 0.00001$). HSP70 expression in the sponge and ascidian

encounters was 2.2 ± 0.7 ng HSP70/μg P, and in all the other encounters (*A. elegantissima* and *C. californica*, calcareous and fleshy algae) the HSP70 level was 1.3 ± 0.6 ng HSP70/μg P.

Discussion

Anthopleura elegantissima and *Corynactis californica* express HSP70 without physical stress (*e.g.*, from temperature, desiccation, changes in pH) or pollution stress (*e.g.*, due to heavy metals, organochlorines). There are few examples of cnidarian HSP expression patterns, and all are directly (Bosch *et al.*, 1988; Bosch and Praetzel, 1991; Sharp *et al.*, 1994) or indirectly (Hayes and King, 1995; Sharp *et al.*, 1997; coral bleaching) related to temperature stress. This is the first set of observations relating aquatic invertebrate HSP levels to biological stress and relating cnidarian HSP expression to parameters other than temperature.

There were significant differences in HSP70 levels between the two cnidarians, and these depended on the particular competing species. Perhaps the aggressive behavior of *C. californica* (Chadwick, 1987, 1991; Chadwick and Adams, 1991) causes cellular damage, thereby increasing HSP70 expression levels in *A. elegantissima* tentacles (Fig. 2) in the first aquarium experiments. *C. californica* extrudes mesentarial filaments upon contact with nonfood species, suggesting that this behavior is used in interspecies aggressive encounters (Chadwick, 1987; Chadwick and Adams, 1991). Prolonged contact with *C. californica* mesentarial filaments kills the competitor. In this forced situation, no stresses other than contact between polyps appear to affect the tentacles of both cnidarians. In comparison with isolated (non-interacting) *A. elegantissima* polyps (Fig. 2), the expression of HSP70 is nearly 20 times greater after 48 h of interspecific interactions. The differences shown between tentacle crown and whole body in *A. elegantissima* were not found in *C. californica*.

The more striking result is the lack of differences between the solitary and interacting *C. californica* polyps in the aquarium experiences (in Fig. 2, compare 24 and 48 h). The expression of HSP70 is high and very constant in the three interspecific encounters (1.3–2.1 ng HSP70/μg P). One explanation could be that the aggressive behavior of some corallimorpharians requires cellular protection to counter the effect of the competing species' response (Chadwick, 1987; Langmead and Chadwick, 1999a, b). After a period of contact with *C. californica*, *A. elegantissima* moved away *via* pedal locomotion, suggesting that the specialized aggressive structures of the anemone were ineffective against the corallimorpharian (Francis, 1973a, b; Chadwick, 1987).

Strong intraspecific competition has been clearly demonstrated between clones of *A. elegantissima* (Francis, 1973a, b; Ayre and Grossberg, 1995, 1996). Contact between genetically different individuals of this species initiates elaborate behaviors involving acrorhagial contact (leaving patches of tissue containing high numbers of nematocysts) and results in damage to one or both competitors. In addition, anemones of the genus *Anthopleura*, including *A. xanthogrammica* (discussed below), produce cytolytic and sodium-channel toxins that presumably damage cellular constituents such as proteins following contact (Bernheimer and Lai, 1985; Cline and Wolowyk, 1997; Kelso and Blumenthal, 1998). These toxic mechanisms could explain the high HSP70 levels found in the examined clones (Fig. 3). The outside warrior polyps bordering neighboring clones have more HSP70 than the inside ones. Sessile organisms discontinuously fight for space, depending on growth and reproductive cycles, the age of competitors, or the nature of the enemies (Connell, 1961; Jackson, 1977; Chadwick, 1991). Perhaps when warrior polyps encounter a "known" competitor (*i.e.*, in this case a different clone of the same species), they become "prepared for war," producing HSP70 levels high enough to avoid serious cellular damage when real interactions begin. Alternatively, some interactions have already caused some tissue damage, resulting in higher HSP70.

No differences in HSP70 expression were expected in interactions between *A. elegantissima* and a fleshy green alga (*Ulva* sp., Fig. 5B). This algal type escapes from direct competition for space by growing as rapidly as nutrients and light levels permit (Lewis, 1964; Paine, 1971). No direct interactions were evident, and the low HSP70 levels found in the outside interacting polyps of these clones seem to confirm their absence, although algae in this genus are capable of producing harmful secondary compounds (Paine, 1990; Whitfield *et al.*, 1999). In the case of *Lithothamnium* sp. (Fig. 5A), it is known that coralline algae grow slowly (Steneck, 1986; Garrabou and Ballesteros, 2000) and can synthesize allelochemicals (as do some other red algae) to compete for space (Whitfield *et al.*, 1999). Perhaps the anemone better detects or is more affected by these *Lithothamnium* chemicals than by those produced by *Ulva*.

A. xanthogrammica is a common intertidal competitor with *A. elegantissima* for space (Francis, 1973b; Sebens, 1984). This solitary anemone elicits aggression in *A. elegantissima* (Francis, 1973b) but does not display the same mechanisms of defense. Observations made by Sebens (1984) support the idea that aggression is common between these two species, which explains the higher levels of HSP70 in the outside *A. elegantissima* polyps in these interactions (Fig. 6A). *Balanus amphitrite*, another common space competitor, seems to have no effect on HSP70 expression (Fig. 6A). It is possible that the lack of effect was due to exposure to small individual cirripeds, and it would be interesting to examine *A. elegantissima* clones that are in competition for space with larger clumps of barnacles.

In *C. californica,* HSP70 levels are similar in outside and inside clone polyps. Therefore the corallimorpharian does not distinguish between the exposed (outside polyps) and nonexposed (inside polyps) areas of the clone. More importantly, even without apparent interactions (Fig. 2), *C. californica* expresses HSP70 at constant levels (1–2 ng HSP70/μg P). In this species, intraspecific competition results in HSP70 levels that are within the "normal" range (Fig. 3), and there is no aggressive behavior in intraspecific contacts (Chadwick, 1987). Perhaps the key to interpreting HSP70 expression as a mechanism of competence in *C. californica* is the finding that the highest HSP70 levels were found in polyps interacting with *Haliclona* or *Synoicum* (Fig. 6B). Also of importance is that these differences between interacting and non-interacting polyps were significant. It is known that sponges and ascidians use chemical substances to defend themselves or attack potential foes competing for substrata (Green, 1977; Suchanek *et al.,* 1985; Thompson *et al.,* 1985; Turon *et al.,* 1996, 1998; Becerro *et al.,* 1997). We suggest that HSP70 expression differences found when the encounter involves ascidians or sponges may reflect the aggressive toxic substances used by these enemies (Uriz *et al.,* 1991).

C. californica appears to be always "prepared for war" by its aggressive behavior (Chadwick, 1991). Another organism that exhibits this strategic use of stress proteins (by maintaining a basal level of HSP expression) is the desert-dwelling ant *Cataglyphys.* This ant presynthesizes HSPs at relatively low nest temperatures to limit damage from heat shock on the desert floor. Coupled with continued HSP production at higher temperatures, this protects the ant from the high temperatures it experiences when foraging in daytime (Gehring and Wehner, 1995). Perhaps the presynthesis of HSP70 in *C. californica* provides protection from neighbors that intermittently excrete harmful substances. Alternatively, the constant HSP70 levels might protect the corallimorpharian against its own aggressive substances, which it uses to catch prey and to fight for space (Chadwick, 1987). The aggressive behavior of *C. californica* includes the extrusion of mesenteric filaments containing gland cells that secrete strong proteolytic enzymes and nematocysts that may inject cytolytic toxins into prey or enemies (Van-Praet, 1985).

Because of the high cost of the HSP expression and its occasional harmful effect if constantly highly expressed (Feder *et al.,* 1992; Krebs and Feder, 1997), we suggest that expression varies depending on the kind of neighboring competitor or enemy. Furthermore, *A. elegantissima* also expresses high levels of HSP70 in response to physical factors, especially temperature (Rossi and Snyder, unpubl. obs.). The anemone has to "share" HSP70 expression between biological (*e.g.,* competition for space) and physical (*e.g.,* temperature) factors.

It is also possible that other stress proteins contribute to the responses against biological phenomena such as competitive interactions for space in the benthic environment. For example, unexpected low-molecular-weight HSP70 homologs have been found in other cnidarians (Sharp *et al.,* 1994). HSP60 has known roles in thermal acclimation of the cnidarians *Hydra vulgaris* and *Acropora grandis* (Bosch *et al.,* 1988; Fang *et al.,* 1997). The use of SPA-822 HSP70 antiserum can possibly underestimate the number of HSP70 isoforms, and consequently may explain the finding of single HSP70 proteins by our methods. However, we have successfully used the same antiserum and measured two and three to four different HSP70 isoforms in larval lobsters, (*Homarus americanus*), and juvenile abalone (*Haliotis rufescens*) and adult mussels (*Mytilus galloprovincialis*) respectively (Snyder and Mulder, 2001; Snyder *et al.,* 2001).

Many questions remain unanswered, such as the identity of the harmful substances or aggressive behaviors that activate HSP70 expression in competitive interactions among sessile marine invertebrates. Among the likely candidates for cellular damaging allelochemicals are cnidarian sodium-channel toxins (Kelso and Blumenthal, 1998), cytotoxic and cytolytic factors (Bernheimer and Lai, 1985; Cline and Wolowyk, 1997), and an array of toxic alkaloids found in cnidarians and sponges (*e.g.,* Djura and Faulkner, 1980; Koh and Sweatman, 2000). Such chemicals can diffuse and act at some distance from the source or can be deposited on neighboring organisms by direct contact (*e.g.,* Schmitt *et al.,* 1995; Slattery *et al.,* 1997). Further studies of HSP proteins may provide important information about the consequent distribution and hierarchy of species in the rocky benthos.

With this work we propose HSP70 expression as a tool for evaluating space competition among sessile marine invertebrates, without manipulative experiments. From our results, it is clear that the expression of the stress proteins depends on both the particular competing species and the interacting life stages of each competitor. The energy required to repair tissue damage cannot be used for other processes such as reproduction and growth. It will be interesting to measure how the amount of energy an organism devotes to growth and reproduction varies with the level of HSP produced during prolonged competition for space.

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